




## Multiparasitism in gills of *Metynnis lippincottianus* from the environmental protection area of Curiaú river, Eastern Amazon

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**ABSTRACT:** The high fish diversity of the Amazon has been the subject of study for several research projects because of the importance of its ecosystems. The Environmental Protection Area of the Curiaú River is composed of permanent and temporary lakes within the floodplain forests, favoring a rich diversity of fish species. *Pratinha* (*Metynnis lippincottianus*) is an ornamental fish, widely distributed throughout Brazil and French Guiana. Fish parasites may reflect the environmental quality, as well as the habits of their host. Considering the importance of understanding and contributing to the expansion of studies on fish parasites, the present study aimed to investigate the gills of *Metynnis lippincottianus* from the Curiaú River basin in the municipality of Macapá (Eastern Amazon). A total of 200 specimens of *Metynnis lippincottianus* from the Curiaú River were examined and 89% of the analyzed fish were parasitized by metacercariae, *Dactylogyridae* gen. sp., *Piscinoodinium pillulare*, *Trichodina* sp., *Henneguya* sp., and *Myxobolus* sp. Despite this high parasitic load, body conditions were not affected. This is the first documented incidence of a species belonging to the Phylum Cnidaria: Myxozoa in *Metynnis lippincottianus*.

**Key words:** ornamental fish, parasitic fauna, infection.

## Multiparasitismo em brânquias de *Metynnis lippincottianus* da área de proteção ambiental do rio Curiaú, Amazônia Oriental

**RESUMO:** A alta diversidade ictiológica da Amazônia tem sido fonte de estudo de diversas pesquisas, por esta região amazônica englobar alguns ecossistemas importantes. A Área de Proteção Ambiental do Rio Curiaú é composta por lagos permanentes e temporários dentro das florestas de várzeas, o que favorece em uma rica diversidade de espécies de peixes. *Metynnis lippincottianus* é um peixe ornamental, amplamente distribuído pelo Brasil e Guiana Francesa. Os parasitos de peixes podem refletir a qualidade ambiental, assim como nos hábitos de seu hospedeiro. Considerando a importância de entender e contribuir para a expansão dos estudos sobre parasitos de peixes, o presente estudo teve como objetivo, investigar as brânquias de *Metynnis lippincottianus* oriundos da bacia do Rio Curiaú no município de Macapá-AP (Amazônia Oriental). Foram examinados 200 exemplares de *Metynnis lippincottianus*, oriundos do rio Curiaú, sendo que 89% dos peixes analisados estavam parasitados por parasitos diversos: *Piscinoodinium pillulare*, *Trichodina* sp., *Henneguya* sp., *Myxobolus* sp., monogenoide da família *Dactylogyridae* e metacercárias. Apesar dessa alta carga parasitária, as condições corporais não foram afetadas. Esta é a primeira ocorrência de espécies do filo Cnidaria: Myxozoa em *Metynnis lippincottianus*.

**Palavras-chave:** peixe ornamental, parasitofauna, infecção.

## INTRODUCTION

The high ichthyological diversity of Amazon has been a subject of study for researchers (TAVARES et al., 2018; DE ANDRADE et al., 2018; ZATTI et al., 2018; BITTENCOURT et al., 2014), but still many issues are need to be studied and understood. (MOREIRA et al., 2010). This geographic

area also encompasses ecosystems that are vital for maintaining the surrounding environment, such as floodplain forests (BATISTA et al., 2015). In the state of Amapá, floodplain forests are the second largest ecosystem in the state, occupy 4.8% of the territory while the rainforest occupies approximately 70% of the state of the Amapá. Since this area is a suitable habitat for many native species (QUEIROZ et al,

2013; PINTO, 2016), 20.83% of these floodplain forest (4,632.71 hectares) are designated as the Environmental Protection Area (APA) of the Curiaú River (LIMA et al., 2013).

The APA of the Curiaú River is composed of permanent and temporary lakes within the floodplain forests, favoring a rich diversity of fish species, such as *Serrasalmus rhombeus* (piranha), *Hoplosternum littorale* (tamoatá), *Cichla temensis* (tucunaré), *Hoplias malabaricus* (traíra), and *Piaractus mesopotamicus* (pacu). Among these, a species of ornamental importance, *Metynnis lippincottianus* (Serrasalminae) known popularly as Pratinha is widely distributed in the Brazilian basins and some French Guiana rivers. It has a diet based on vegetables, seeds, phytoplankton, mollusks and some arthropods and detritus (MOREIRA et al., 2009; HOSHINO et al., 2014).

In the Amazon, several factors influence the parasitic load of fish, such as seasonality, abiotic and biotic factors in aquatic environment water, and host ecology (NEVES et al., 2013). The diversity and ecological function of the parasites in an ecosystem can be used as tools for a better understanding of the biosphere, as well as the parasitic indexes that support parasite-host relationship analysis (TAVARES-DIAS et al. 2014 CARDOSO et al., 2018). TAKEMOTO et al. (2004) stated that all fish hosted at least one species of parasite and the location of the parasites may vary, where no organ is free from parasitism, but one organ may have more parasites than another organ.

Multiple studies have reported that gills are one of the most parasitized organs (CARDOSO et al., 2018; JERÔNIMO et al., 2014, GONÇALVES et al., 2014, VENTURA et al., 2013, SANTOS et al. 2013; OLIVEIRA et al., 2016). Gills are easily damaged by parasitic infections, being the first organ in contact with the external environment. Additionally, gills perform several functions, such as respiration, osmoregulation, and excretion. Gills are an indicator of the rate of parasitism, based on histopathological changes, such as respiratory disorders and electrolyte imbalance (FLORES-LOPES et al., 2011; NASCIMENTO et al., 2012). Fish parasites may reflect the environmental quality, as well as habits of their host, considering the importance of understanding and contributing to the expansion of studies on fish parasites (FALKENBERG et al., 2019). Therefore, the present study aimed to investigate the gills of *Metynnis lippincottianus* from the Curiaú River basin in Macapá (eastern Amazon).

## MATERIALS AND METHODS

### Study area

The Curiaú river basin measures approximately 584.47 km<sup>2</sup>, almost 40% of the Curiaú River's Environmental Protection Area (APA) (LIMA et al., 2013). The Curiaú River's mouth in the Amazon River presents meandric characteristics, which may be due to the greater turbulence in the river, caused by the speed of the water current and the Amazonian river tidal regime (VASCONCELOS et al., 2011).

Specimens of *M. lippincottianus* (COPE, 1870), common fish species of the Curiaú River (Point 1: 51°2'57,205" W 0°8'29" N; Point 2: 51°2'30",743 W 0°8'43,087" N), were collected during a 12-month period, from August 2017 to August 2018. Collections were carried out twice per month for parasitological analysis, using a 20 mm net between knots measuring five meters long and two meters high, being placed in points with intense activity of fishing by the local population (Figure 1).

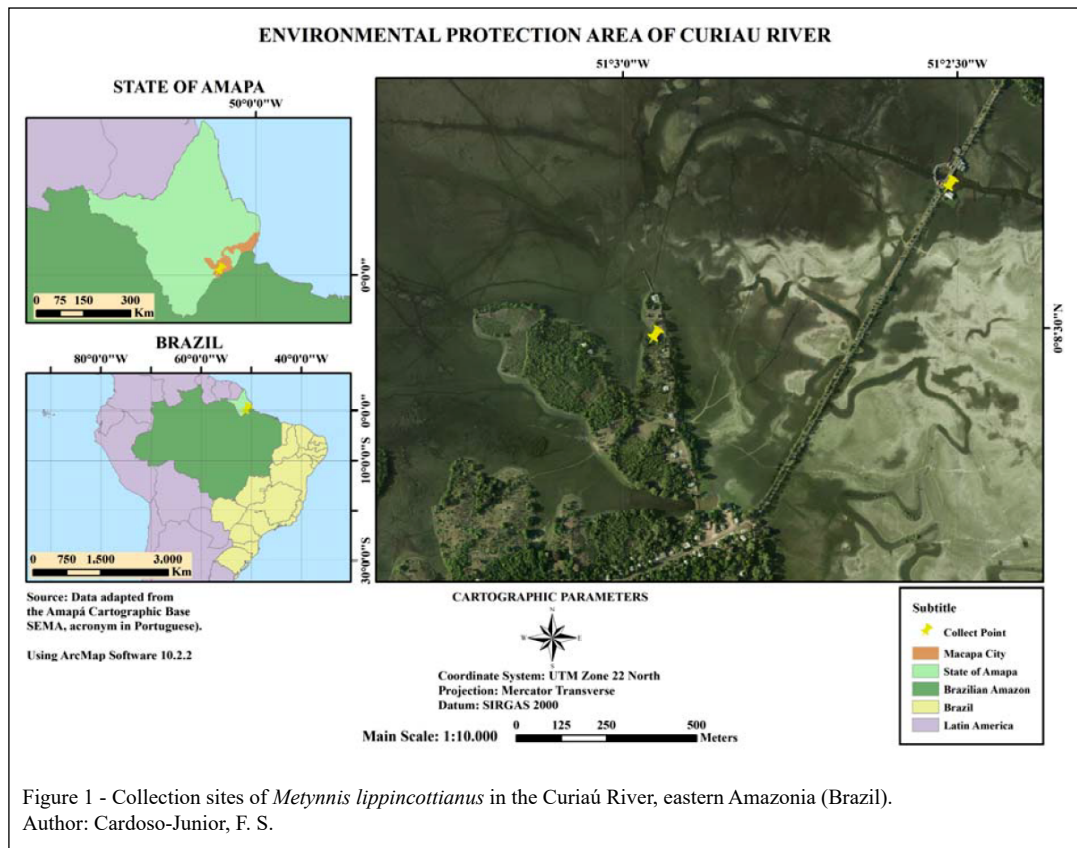
### Parasites sampling procedures

All fish were transported alive in vats containing water from the environment and artificial aeration, to the Laboratory of Morphophysiology and Animal Health (LABMORSA) at the State University of Amapá (UEAP). The specimens were desensitized through a medullary incision, using pincers and a scalpel. Then, biometric data such as total length (cm), standard length (cm) and weight (g) were measured.

The entire external surface, mouth, nostrils, fins, and gills were analyzed using stereoscopic binocular microscopes, to verify the existence of parasites, cysts, or lesions. During the necropsy, small gills fragments were separated between slides and coverslips, wherein foci of parasite development were identified via light microscope analysis.

Prevalence was used to analyze the infection level of the parasites following the recommendations of BUSH et al. (1997). A relative condition factor for the host was determined using body weight (g) and total length (cm) data following LECREN (1951) where the expected and observed weight are used to calculate, which has a value equal to one ( $K_n=1$ ) under normal conditions.

The project was submitted to the Ethics Committee for Animal Use (CEUA), nº 012-CEUA/CPAFAP and to the System of Authorization and Information on Biodiversity (SISBIO) nº 50376-1. A license was also obtained from the Secretary of the Environment of the State of Amapá (SEMA-AP)



(letter nº 1014 / 2016), due to the status of the research site as an Environmental Protection Area.

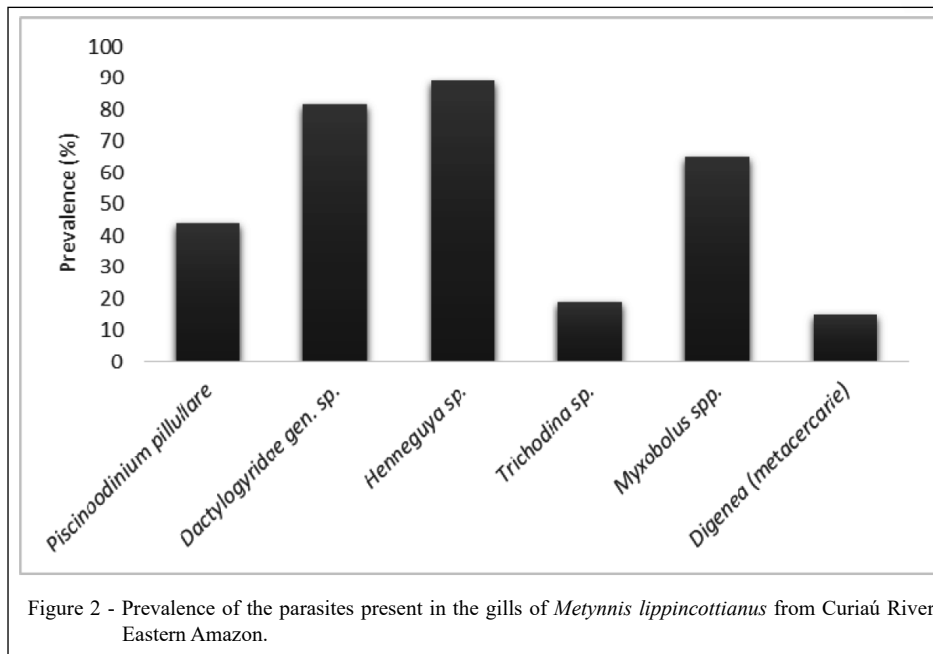
## RESULTS AND DISCUSSION

The 200 specimens of *M. lippincottianus* examined from the Curiaú River, had a mean total length of  $7.77 \pm 0.78$  cm; standard mean length of  $6.21 \pm 0.64$  cm; and mean weight of  $9.17 \pm 2.82$  g. Eighty-nine percent were parasitized by one or more species. Three taxa (Ciliophora, Cnidaria and Platyhelminthes) and 6 groups of parasites: *Piscinoodinium pillulare*, *Trichodina* sp., *Henneguya* sp., *Myxobolus* sp., monogenoids from the family Dactylogyridae, and unidentified digenetics (metacercariae) (Figure 2). *Cnidarian Henneguya* sp. presented the highest prevalence (89%) among the parasites reported in gills; metacercariae showed the lowest prevalence (15%).

The dinoflagellata *Piscinoodinium pillulare* was found in the gills of 44% of the analyzed *M. lippincottianus* specimens, which was lower than the prevalence reported by FLORINDO

et al. (2017) in ornamental fish from Santa Catarina, which was 75% in all fish. This same parasite was reported in *Cichlasoma amazonarum* and in *Hemibrycon surinamensis* of the Igarapé of Fortaleza basin, Macapá, with a prevalence of 49% and 17.2%, respectively (CARVALHO et al., 2017; HOSHINO et al. 2014; HOSHINO et al., 2014). *Piscinoodinium pillulare* is common in cold season of the year and is responsible for outbreaks in aquaculture, which may cause discomfort and asphyxia in hosts, as described by SANT'ANA et al (2012).

Parasitic infection of the gills in the genus *Trichodina* is the main cause of mortality among fish farms (MACIEL et al., 2018). In this study, the prevalence of *Trichodina* sp. in the gills was 19%, which was higher than the prevalence (10.4%) reported by NEVES et al. (2013) in *Astronotus ocellatus* from Pracuúba Lake, Amapá. *Trichodina* spp. was found in gills of *Carnegiella strigata*, *Carnegiella mariae*, and *Nannostomus eques*, with a prevalence of 14.3%, 7.9%, and 9.7%, respectively; all of these fish were collected from the middle Rio Negro (TAVARES-DIAS et al.,



2010). *Trichodina nobilis* parasitized the gills in 64.3% and 84.2%, respectively, of in *Pterophyllum scalare* and *Mesonauta acora* individuals (FARIAS PANTOJA et al., 2015).

In the present study, the parasite that presented the highest prevalence of infection was *Henneguya* sp., which infected 89% of the specimens analyzed. This prevalence was greater than that reported in *Hypophthalmus marginatus* of the municipality of Cametá, in the state of Pará, in which 80% of the individuals were parasitized by *Henneguya* sp. (VELASCO et al., 2015), this higher prevalence can be related directly as the feeding habit of the fish species, as well as the behavioral, biological and physiological differences of these fish (ISAAC et al., 2004) because these factors can affect the structure of the parasite community (CARVALHO et al., 2017). *Henneguya* sp. was also described in *Pimelodus maculatus*, infecting 13.4% of the gills (MARTINS et al., 2018). *Henneguya paraensis* was reported in 60% of the gills of *Cichla temensis* specimens studied (VELASCO et al., 2016). *Henneguya aequidens* occurred in 33.3% of the gills of *Aequidens plagiozonatus* individuals (VIDEIRA et al., 2015); whereas in *Arapaima gigas*, *Henneguya arapaima* parasitized the gill arches and gall bladder with a prevalence of 11.7% and 82.3%, respectively (FEIJÓ et al., 2008). This parasite has high specificity for its host fish, and its parasitic action brings not only

ultrastructural damages that can result in death, but also cause sterility of the host when housed in the gonads and testicles (MATOS et al., 2001).

*Myxobolus* spp. parasitized 65% of gills of the fish examined, a value higher than that reported in the heart of *Pimelodus ornatus*, from the Arari Waterfall, which had a prevalence of 13.9% in the 43 specimens analyzed (MATOS et al., 2014). LACERDA et al (2013) explained that the discrimination of the fish parasitic fauna can be based on premise that the different biogeographic regions showed a range of possibilities for the parasitic fauna structure in the host, thus explaining the differences in parasites prevalence in their hosts. A species of *Myxobolus*, *Myxobolus maculatus*, was found to parasitize 40% of the kidneys of *Metynnis maculatus*, a fish of the same genus as those researched in this study, collected in the Amazon River estuary (CASAL et al., 2002). *Myxobolus insignis* infected the gills of 66.6% of *Semaprochilodus insignis* (EIRAS et al., 2005) and *Myxobolus* sp. of 5.5% of *Colossoma macropomum* (MACIEL et al., 2011), both fish species being from the Amazon basin. *Myxobolus marajoensis*, was found to parasitize the intestinal musculature of 20% of fish from Paracauri River, in the Island of Marajó-PA, *Rhamdia quelen* (ABRUNHOSA et al., 2017).

Monogenoids of the family Dactylogyridae presented the second highest prevalence, at 81.5% of

the specimens examined. HOSHINO & TAVARES-DIAS (2014) described the presence of a species of the family Dactylogyridae in *M. lippincottianus* of the Igarapé Fortaleza basin / AP. The species reported was *Anacanthorus jegui*, with a prevalence of 95% in the 80 specimens analyzed. In another study, conducted by REVERTER et al. (2016) in gills of butterfly fishes of the Tropical Islands of the Western Pacific, parasitism by monogenea communities of the Dactylogyridae family to occurred with prevalences between 40% and 100% in the analyzed species; members of the family Dactylogyridae are thus present in both freshwater and marine environments. According to MENDOZA-FRANCO et al. (2018), the occurrence of monogenea in ornamental freshwater fish is due to the introduction of exotic fish that harbor these parasites and the pollution of the natural environment.

The presence of digenetic metacercariae larvae in the gills of *M. lippincottianus* was observed with the lowest prevalence among the parasites reported—only 15% of the individuals examined were infected. It was not possible to identify the individual species of metacercariae. In studies conducted in Lago Guaíba / RS, 13 species belonging to the phylum Platyhelminthes were parasitizing the intestines, gills, and stomach of *Megaleporinus obtusidens*, with prevalence between 1.66% and 86.66% in the 60 specimens analyzed (WENDT et al., 2018). There were metacercariae of *Posthodiplostomum* sp. parasitizing the gills of *Auchenipterus nuchalis* (TAVARES-DIAS, 2017) and metacercariae in the gills of 77.5% of *M. lippincottianus* (HOSHINO et al., 2014); both fish species distributed the Igarapé of Fortaleza basin. MORAIS et al., (2011) reported metacercariae of *Clinostomum marginatum* and *Austrodiplostomum compactum*, parasitizing 100% and 15%, respectively, present in *Pygocentrus nattereri* of central Amazon.

The relative factor of the host ( $Kn=1.000\pm 0.08$ ) was not affected by the parasitism, since it remained very close to the standard value, that is  $Kn=1.0$ , according to LECREN (1951), thus indicating that the specimens' condition was not impacted, despite the high parasitic load. This relative condition factor indicated the well-being of the fish, thus measuring the state of animal health (VAZZOLER, 1996, LIZAMA et al., 2006). FALKENBERG et al. (2019) said that under natural conditions, fish can be infected by many species, which coexist and show interrelations, demonstrating that each host has its own community of parasites and that hosts acquire resistance, as well as adapt with the

presence of parasites and thus not have a negative influence on the condition factor.

## CONCLUSION

The parasitic fauna of the gills of *M. lippincottianus* comprised micro and macroparasites, was diverse in its composition, and was dominated by the phylum Cnidaria: Myxozoa. *Henneguya* sp. was the most prevalent parasite, while metacercariae were the least prevalent. The presence of protozoa, *Trichodina* sp. and *Piscinoodinium pillulare*, occurred in almost 50% of the specimens, and these are primarily responsible for production losses in aquaculture. The presence of two species of the genus *Myxobolus* was observed, which were differentiated based on spores of different shapes, since species identification for myxozoa requires molecular and ultrastructural analysis. This is first observed occurrence of species belonging to the phylum Cnidaria: Myxozoa in *M. lippincottianus*.

## DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

## BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This research was approved by the Animal Use Ethics Committee (CEUA) of EMBRAPA-AP, number 012-CEUA/CPAFAP and registered in the System of Authorization and Information of Biodiversity (SISBIO), nº 50376-1, as well as authorized by the Secretary of Environment of the State of Amapá (SEMA), for the reason of the study being conducted in Environmental Protection Area, under the official number 1014/2016.

## AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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